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Identification of *Magnaporthe oryzae* Avirulence Gene Corresponding to the Rice Blast Resistance Gene *Pik-m*

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Summary

To identify a blast fungus avirulence (*Avr*) gene that correspond to the rice blast resistance (*R*) gene *Pik-m,* the difference between the *Avr* gene in the parental isolate (84R-62B) and the *Avr* gene in the standard isolate (Ken54-20) harboring known *Avr* genes was investigated by using 187 lines of F_3 family from the cross of rice cultivars Norin 3 (+) and Tsuyuake (*Pik-m*). The pathogenic reactions of the two isolates 84R-62B and Ken54-20 were always identical on all 187 lines without exception. This indicates that the isolate 84R-62B has the same *Avr* gene as the isolate Ken54-20 has. Moreover, it appears that the incompatibility between the cultivar Tsuyuake and the isolate Ken54-20 is determined by only the interaction of the *R* gene *Pik-m* and the *Avr* gene corresponding to *Pik-m.* As a result, *Avr* gene of the isolate 84R-62B to Tsuyuake was identified as the *AvrPik-m.* The segregation ratio of avirulence/virulence in progeny isolates was 72 : 43 on Tsuyuake, which does not fit a 1 : 1 or a 3 : 1 ratio expected from the involvement of one or two independent aviruence gene (s). On the other hand, the resistance reaction of F_3 rice lines to the isolate 84R-62B was consistent with a 1 : 2 : 1 ratio, indicating that the blast resistance of Tsuyuake is due to monogenic control. A particular interaction of the *R* genes *Pik-m* and *Pik* with the corresponding *Avr* genes in the rice blast fungus was discused herewith.

Key words: rice disease, blast fungus, avirulence gene

Introduction

An ascomycetous fungus *Magnaporthe oryzae*[1] (formerly *M. grisea*) is one of the most important pathogens of rice (*Oryza sativa*) because of its world wide distribution and the cause of severe yield loss. Growing resistant cultivars of rice is the most effective and economic way to control the blast disease. However, the newly developed resistant cultivars often give way to blast fungus after a few years of commercial production. Studying a genetic interaction between the avirulence (*Avr*) gene in blast fungus and the resistance (*R*) gene in rice is very important for understanding how this pathogen overcomes the resistance of rice. Although the breakdown of resistance in rice cultivers to blast is probably due to genetic instability of *Avr* genes, there is only limited information on the *Avr* genes in this pathogen corresponding to the *R* genes in rice[7, 11, 12, 14, 15]. To identify the *Avr* gene that correspond to the specific *R* gene *Pik-m*[10], studies were conducted based on the reaction of F_3 family from a cross of rice cultivars Norin 3 and

Tsuyuake to both the parental isolate and the standard isolate harboring known *Avr* genes[9]. We also discussed herewith a particular interaction of the *R* genes *Pik-m* and *Pik* with the corresponding *Avr* genes in the rice blast fungus.

Materials and Methods

1. Pathogen

Rice blast fungus used in this study are shown in Table 1. The 84R-62B, one of parental isolates, which has avirulence to rice cultivar Tsuyuake was isolated in 1984 in Aichi prefecture. The standard isolate Ken54-20, which was used by Kiyosawa[9] for determining the blast *R* gene in rice cultivar Tsuyuake, were obtained from the National Agricultural Research Center for Kyushu Okinawa Region. An isolate F1-354 obtained from a cross of two field isolates[11,12] was used as a particular genotypic one that has virulence on Kanto 51 (*Pik*) but avirulence on Tsuyuake (*Pik-m*).

2. Host

Rice cultivars Norin 3 and Tsuyuake were used in this study. Tsuyuake was used as *R* gene donor in developing F_3 lines of rice for genetic analysis of the *Avr* gene in the rice blast fungus. In the Norin 3/Tsuyuake family, the blast resistant cultivar Tsuyuake was used as the pollen parent in a cross with the susceptible cultivar Norin 3 at the National Agricultural Research Center, Joetsu. Randomly selected F_1 seeds were grown in a field and self-pollinated to obtain F_2 seeds. The F_2 seeds were randomly chosen and planted in a paddy field of Saga University. The F_3 seeds were harvested from each individual plant and established as F_3 lines, i.e., self-pollinated descendants from single F_2 seeds. The F_3 family of rice consisted of 187 lines in all. Cultivar Norin 3 was also used as a susceptible control in each inoculation test.

Seeds were germinated prior to planting to ensure uniform emergence and facilitate disease

Table 1. Rice blast isolates used and their pathogenic reaction on Japanese race-differentials and Norin 3

		Rice blast isolate[b]		
Code No.[a]	Resistance genotype	84R-62B	Ken54-20	F1-354
1	*Pik-s, Pish, Pi 19(t)*	S	S	S
2	*Pia, Pi 19(t)*	S	S	S
4	*Pii, Pik-s, Pi 19(t)*	S	R	S
10	*Pik, Pi 19(t)*	R	R	S
20	*Pik-m, Pi 19(t)*	R	R	R
40	*Piz, Pish, Pi 19(t)*	S	R	R
100	*Pita,*	R	R	S
200	*Pita-2, Pish*	R	R	R
400	*Piz-t, Pish, Pi 19(t)*	S	R	S
Norin 3[c]	+	S	S	S
(Race)		(447)	(003)	(517)

a) Code No.1, 2, 4, 10, 20, 40, 100, 200 and 400 of race-differential rice cultivars: Shin 2, Aichi Asahi, Ishikari Shiroke, Kanto 51, Tsuyuake, Fukunishiki, Yashiro-mochi, Pi-No.4 and Toride 1, respectively.

b) R: resistant reaction, S: susceptible reaction.

c) Rice cultivar Norin 3, which has no resistance gene to blast fungus, was used as the susceptible parent to make an F_3 line of rice.

ratings. For *Avr* gene identification of the parental isolate 84R-62B, about twenty seedlings per rice line were grown in the seedling boxes (5×15×10 cm).

3. Inoculation and evaluation

Blast isolates were grown on oatmeal agar (1 liter of distilled water, 50g of ground oats, 20g of sugar, and 20g of agar) for 14 days at 25 to 27 ℃. Then the surface of the oatmeal agar culture in petri dishes was rubbed with a wet paintbrush to remove aerial mycelia, then exposed to fluorescent light for 3 days at 25 ℃ to induce sporulation. The cultures were flooded with distilled water containing 0.01% Tween 20, and conidia were dislodged with a paintbrush. The conidial suspension was poured through Kimwipe paper (Crecia, Tokyo, Japan) and adjusted to approximately 2.0 to 5.0×10^5 spores per ml. Rice seedlings at the four-leaf stage were sprayed with 100 ml of the spore suspension per 16 seedling boxes. Inoculated plants were incubated in a moist chamber for 18 to 20 hr at 25 to 27 ℃ and then returned to the greenhouse. The reactions of the rice lines were recorded 7 days after inoculation. Inoculation tests were repeated two or three times. The disease reaction scale used was as follows: 0= no lesions; 1= brown pinpoint lesions; 2 = less than 2 mm brown lesions, sometimes with gray center; 3= circular lesions with gray centers, lesion size variable; 4= necrotic eyespot lesions with gray centers. Plants with reaction types 0, 1 and 2 were considered resistant, whereas reaction types 3 and 4 were considered susceptible.

4. Nomenclature

The term avirulence was applied to an interaction that was demonstrated to be cultivar specific. An *Avr* gene, for which an allele determines avirulence on a specific rice cultivar, was named *Avr* with a symbol referring to the particular *R* gene of the cultivar on which the gene was effective (e.g., *AvrPik*).

Results and Discussion

Since a gene-for-gene relationship[3,4] has been proposed between the *R* genes in *Oryza sativa* and *Avr* genes in *Magnaporthe oryzae*[7,9,13], the *Avr* genes could be predicted from the existence of corresponding *R* genes in rice cultivars. Based upon the resistance reactions of the F_3 rice lines to the parental isolate and the standard isolate harboring known *Avr* genes, the *Avr* genes *AvrPik, AvrPiz* and *AvrPiz-t* had been identified in a previous study of the rice blast isolate[11]. However, an *Avr* gene corresponding to the *R* gene *Pik-m* has not been identified yet, although we had discussed tentatively on the relationship between the *Avr* genes of the same family, *AvrPik* and *AvrPik-m*[12]. To identify the *Avr* gene that correspond to the *R* gene *Pik-m,* the difference between the *Avr* gene in the parental isolate 84R-62B and the *Avr* gene in the standard isolate Ken54-20 was investigated by using the 187 lines of F_3 family from the cross of the rice cultivars Norin 3 and Tsuyuake. As shown in Table 2, the pathogenic reactions of the parental isolate 84R-62B and the standard isolate Ken54-20 were always identical on all 187 lines. That is, each resistant line, segregating line, or susceptible line to the isolate 84R-62B was always resistant, segregating, or susceptible to the isolate Ken54-20 without exception. This indicates that the isolate 84R-62B has the same *Avr* gene as the isolate Ken54-20 has, as far as the data on the pathogenic reaction is concerned. On the other hand, the isolate Ken54-20 had been identified to have *Avr* genes corresponding to *R* genes *Pii, Pik, Pik-m, Piz, Pita, Pita-2* and *Piz-t,* respectively[9]. Moreover, it

Table 2. Reaction of individual F_3 lines of rice to parental and the standard isolates of *Magnaporthe oryzae*[a]

Standard isolate	Reaction to Ken 54-20[b]	Number of F_3 lines classified by reaction to 84R-62 B				χ^2	
		Resistant	Segregating	Susceptible	(Total)	1:2:1	*P*
Ken54-20	Resistant	45	0	0	(45)		
	Segregating	0	101	0	(101)		
	Susceptible	0	0	41	(41)		
	(Total)	(45)	(101)	(41)	[187]	1.37	0.5

a) The F_3 lines of rice from the cross between Norin 3 (+) and Tsuyuake (*Pik-m*).

b) Resistant (or susceptible): all of individual plants in a given F_3line showed resistant (or susceptible) reaction to the blast isolate used. Segregating: host reaction to the blast isolate segregated depending on individual plants of the same rice line.

Table 3. Reaction of individual F_3 lines of rice to parental and the selected progeny isolates of *Magnaporthe oryzae*[a]

Reaction to F_1-354[b]	Number of F_3 lines classified byreaction to 84R-62B				χ^2	
	Resistant	Segregating	Susceptible	(Total)	1:2:1	*P*
Resistant	45	0	0	(45)		
Segregating	0	101	0	(101)		
Susceptible	0	0	41	(41)		
(Total)	(45)	(101)	(41)	[187]	1.37	0.5

a) The F_3 lines of rice from the cross between Norin 3 (+) and Tsuyuake (*Pik-m*).

b) Resistant (or susceptible): all of individual plants in a given F_3line showed resistant (or susceptible) reaction to the blast isolate used. Segregating: host reaction to the blast isolate segregated depending on individual plants of the same rice line.

appears that the incompatibility between the cultivar Tsuyuake and the isolate Ken54-20 is determined by only the interaction of the *R* gene *Pik-m* and the *Avr* gene corresponding to *Pik-m,* because of the evidence that Tsuyuake has two *R* genes *Pik-m* and *Pi 19(t)*[6,10] but both of the isolates 84R-62B and Ken54-20 have no *Avr* gene corresponding the *R* gene *Pi 19(t)*[11]. As a result, *Avr* gene of the isolate 84R-62B to Tsuyuake was identified as the *AvrPik-m.*

In previous paper[12], we had reported a particular relationship between the *Avr* genes *AvrPik* and *AvrPik-m* of the same family, and presumed that the *AvrPik-m* consisted of at least two genes and one of which was *AvrPik* itself. Therefore, it needs here to distinguish between the genes *AvrPik-m* and *AvrPik* more precisely. To confirm the *Avr* gene of the parental isolate 84R-62B, a particular progeny isolate F1-354, which has virulence on Kanto 51 but avirulence on Tsuyuake, were used additionally and investigated the resistance reaction of F_3 rice lines to both of the parental isolate and the particular progeny isolate. As shown in Table 3, a complete cosegregation for resistance to both of the isolates was observed in the F_3 lines from rice cross. It means that the isolate 84R-62B surely has the *Avr* gene corresponding to the *R* gene *Pik-m.* However, the segregation ratio of avirulence/virulence in progeny isolates was 72 : 43 on Tsuyuake as indicated in a previous paper[12]. It does not fit a 1 : 1 or a 3 : 1 ratio expected from the involvement of one or two independent avirulence gene (s), although the avirulence on cultivar Kanto 51 (*Pik, Pi 19(t)*) of the same family in blast resistance gene is under monogenic control[11,12]. Interestingly, all progeny isolates avirulent to Kanto 51 were avirulent to Tsuyuake without exception, but progeny iso-

lates virulent to Kanto 51 consisted of a few avirulent isolates (recombinant type) and a fairly large number of virulent isolates (parental type) on Tsuyuake[12]. One possible genetic explanation is that the gene *AvrPik-m* is a so-called complex locus consisting of two or more closely linked genes which is functionally related, i.e., *AvrPik-m* consists of at least two genes *AvrPik-m1* and *AvrPik-m2,* each of which has a function in the whole gene *AvrPik-m,* and that one of them is *AvrPik.*

On the other hand, in the resistance reaction of the Norin 3/Tsuyuake family, the segregation ratio of 187 rice lines to the parental isolate 84R-62B was 45 all resistant : 101 segregating for resistance : 41 all susceptible. This segregation was consistent with a 1 : 2 : 1 ratio, indicating that the resistance of cultivar Tsuyuake to isolate 84R-62B is due to monogenic control.

However, it is open to question how a single *R* gene responds to two *Avr* genes. Assuming that the *R* gene *Pik-m* consists of at least two genes *Pik-m1* and *Pik-m2* which are tightly linked and one of them is the *R* gene *Pik,* we could account for the specific interaction between the *R* gene *Pik-m* and the *Avr* gene *AvrPik-m.* Because the *R* genes *Pik-m1* and *Pik-m2* are not segregated in at least 187 F_3 rice lines, the inheritance pattern of the *R* gene *Pik-m* (= *Pik-m1* and *Pik-m2*) seems to be a "single" gene. On the contrary, the *Avr* gene *AvrPik-m* consisted of two or more genes linked at the recombination value of ca. 8.7%. It was confirmed by the recovery of recombinant phenotypes in progeny isolates.

The *R* gene *Pik-m* has been reported as inseparable in the 118 F_3 individuals from the cross of rice cultivars Tsuyuake and Shin 2[10]. This monohybrid segregation of *Pik-m* was consistent with our result. Kiyosawa also reported that the defense spectrum of *Pik-m* against the blast fungus always includes that of *Pik*[9]. Although the *R* gene in cultivar Tsuyuake was designated firstly as *Pim*[8], the siymbol of this gene was renamed as *Pik-m* for *Pik* and *Pim* because the two genes were inseparable[8, 10]. These results suggest that the *R* gene *Pik-m* in cultivar Tsuyuake includes the *R* gene *Pik* in it.

Summing up the above mentioned results, the following conclusions could be suggested: i) the *R* gene *Pik-m* in cultivar Tsuyuake contains at least two genes *Pik-m1* and *Pik-m2,* and one of which is *Pik*; ii) *Pik-m1* was tightly linked with *Pik-m2* and inseparable with each other in genetic analysis. Finally, it should be noted that the possibility of *Pik-m* as a "single" gene could not be ruled out completely based on genetic analysis only, if the *R* gene *Pik-m* could confer multiple specificity to isolates expressing either of two *Avr* genes *AvrPik-m1* and *AvrPik-m2.* The *Arabidopsis RPM1* gene has been shown to respond to two distinct *Pseudomonas syringae Avr* genes, *avrB* and *avrRpm1*, demonstrating that *R* genes may have multiple specificities[2, 5].

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イネいもち病抵抗性遺伝子 *Pik-m* に対応する非病原性遺伝子の同定

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摘　　要

イネいもち病抵抗性遺伝子*Pik-m*に対応する非病原性遺伝子を同定するため，交配親菌株84R-62Bおよび標準菌株Ken54-20のイネF_3系統に対する病原性反応を比較した．イネ農林3号(+)とツユアケ（*Pik-m*）のF_3系統（187系統）に対する交配親菌株84R-62Bの反応が，既知の非病原性遺伝子を有する標準菌株Ken54-20のそれと同じであるか否かによって，交配親菌株の非病原性遺伝子を同定しようとするものである．両菌株のイネF_3系統に対する病原性反応は187系統のいずれにおいても例外なく一致した．これは84R-62BとKen54-20が共に同じ非病原性遺伝子を有することを意味している．ツユアケとKen54-20間の非病原性は，抵抗性遺伝子*Pik-m*と非病原性遺伝子*AvrPik-m*との相互作用によると考えられるので，ツユアケに対する84R-62Bの非病原性遺伝子は*AvrPik-m*と同定された．交配子孫菌株のツユアケに対する病原性反応（非病原性：病原性）の分離比は72：43で、1遺伝子支配あるいは2遺伝子支配の場合に想定される分離比1：1あるいは3：1のどちらにも適合しなかった．一方，菌株84R-62Bに対するイネF_3系統（187系統）の抵抗性反応は1：2：1の分離比に適合した．これはツユアケのいもち病抵抗性には1つの抵抗性遺伝子が関与していることを意味している．2つの抵抗性遺伝子*Pik-m*および*Pik*とそれに対応する非病原性遺伝子との特殊な相互関係について議論した．